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# *Lecanicillium cauligalbarum* sp. nov. (Cordycipitaceae, Hypocreales), a novel fungus isolated from a stemborer in the Yao Ren National Forest Mountain Park, Guizhou

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## Abstract

A new species of entomopathogenic fungi, *Lecanicillium cauligalbarum*, was discovered from a survey of invertebrate-associated fungi in the Yao Ren National Forest Mountain Park in China. The synnemata of this species emerged from the corpse of a stemborer (Lepidoptera), which was hidden amongst pieces of wood on the forest floor. It differs from morphologically similar *Lecanicillium* species mainly in its short conidiogenous cells and ellipsoid to ovoid and aseptate conidia. Phylogenetic analysis of a combined data set comprising ITS, *SSU*, *LSU*, *TEF*, *RPB1* and *RPB2* sequence data supported the inclusion of *L. cauligalbarum* in the *Lecanicillium* genus and its recognition as a distinct species.

## Keywords

Entomopathogenic fungi, *Lecanicillium*, multiple genes, phylogeny, new species

## Introduction

The entomopathogenic fungal genus *Lecanicillium* W. Gams & Zare belongs to Ophiocordycipitaceae. It is typified by *Lecanicillium lecanii* with *Torrubiella confragosa* as the sexual morph (Zare and Gams 2001, Wijayawardene et al. 2017). *Lecanicillium lecanii*

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was first named as *Cephalosporium lecanii* Zimm. by Zimmermann in 1898. Viegas incorporated the species in *Verticillium* Nees in 1939 (Gams and Zare 2001). The genus *Verticillium* has a wide host range, including arthropods, nematodes, plants and fungi (Goettel et al. 2008). Zare and Gams (2001) recircumscribed the genus following analyses of morphological data and sequence data for the internal transcribed spacer (ITS) rDNA region (which comprises the ITS1 spacer, 5.8S coding region and ITS2 spacer). All insect pathogens formerly included in *Verticillium* were reclassified in a newly established genus, *Lecanicillium*. In more recent studies, a multilocus nuclear DNA dataset combining sequence data for the nuclear small subunit rDNA (*SSU*), nuclear large subunit rDNA (*LSU*), translation elongation factor 1α (*TEF*), DNA-dependent RNA polymerase II largest subunit (*RPB1*) and DNA-dependent RNA polymerase II second largest subunit (*RPB2*) genes suggests that the genus *Lecanicillium* is paraphyletic (Sung et al. 2007). Phylogenetic analysis of ITS sequence data also supports this conclusion (Sukarno et al. 2009). Kepler et al. (2017) revisited the taxonomic affinities of the Cordycipitaceae (Hypocreales) and proposed that *Lecanicillium* should be rejected because *L. lecanii* is included within the *Akanthomyces* clade and the name *Akanthomyces* Lebert has nomenclatural priority over *Lecanicillium* (Kepler et al. 2017). However, Kepler et al. (2017) transferred to *Akanthomyces* only several species for which sufficient information was available. The phylogenetic affinities of the majority of species in the original circumscription of *Lecanicillium* remain uncertain. Given that there remain unresolved phylogenetic and taxonomic matters concerning *Lecanicillium*, Huang et al. (2018) and Crous et al. (2018) chose to describe new taxa in *Lecanicillium* to avoid creating further confusion in the taxonomy (Crous et al. 2018; Huang et al. 2018).

Presently, 29 *Lecanicillium* species have been formally described and are listed in the Index Fungorum (http://www.indexfungorum.org). Zare and Gams (2001) recognised 14 *Lecanicillium* species based primarily on morphology and ITS sequence data (Zare and Gams 2001). Subsequently, an additional five new *Lecanicillium* species, based on ITS sequence data, were described (Kope and Leal 2006, Sukarno et al. 2009, Kaifuchi et al. 2013). In order to add more sequence information with ITS, Zare and Gams (2008) reassessed the genus *Verticillium* and transferred four species to *Lecanicillium* based on ITS and *SSU* sequence data (Zare and Gams 2008). Except for the *SSU* and ITS gene, more and more researchers have labelled the *Lecanicillium* genus by *TEF* gene. Based on this, two new *Lecanicillium* species were confirmed based on combined with ITS and *TEF* sequence data (Crous et al. 2018). With combined multigene identification of species gradually becoming the convention, two new *Lecanicillium* species were identified based on multilocus (*TEF*, *RPB1*, *RPB2*, *LSU* and *SSU*) sequence data (Park et al. 2016, Chen et al. 2017). *Lecanicillium sabanense* was identified based on phylogenetic analysis of combined multilocus and ITS sequences (Chiriví-Salomón et al. 2015). *Lecanicillium subprimulinum* was identified based on combined analysis of *LSU*, *SSU*, *TEF* and ITS sequence data (Huang et al. 2018).

We carried out a survey of invertebrate-associated fungi in the Yao Ren National Forest Mountain Park near Sandu county in Guizhou province, China. A parasitic

fungus was found on a stemborer (Lepidoptera) hiding amongst pieces of wood. Attempting to identify the fungus, we determined it to be a member of *Lecanicillium* but its morphological traits and gene sequences did not correspond with those of any known *Lecanicillium* species. On the basis of its morphology and molecular phylogenetic analysis of multilocus nuclear genes (*TEF*, *RPB1*, *RPB2*, *LSU* and *SSU*) and ITS sequence data, this fungus was suggested to be an unnamed species of *Lecanicillium* and is here described and named *Lecanicillium cauligalbarum* sp. nov.

## Materials and methods

### Specimen collection and fungus isolation

The specimen was collected from Yao Ren National Forest Mountain Park, Sandu county, Guizhou, China (107°53', 107°58'E; 24°54', 25°59'N, approximately 560–1365 m above sea level), in September 2015 by Yeming Zhou and Xiao Zou. The synnemata of this species emerged from a dead stemborer (Lepidoptera) hidden amongst pieces of wood on the forest floor. The specimen GZUIFR–2015ZHJ and two isolated strains of the fungal asexual stage, GZUIFRZHJ01 and GZUIFRZHJ02, were deposited at the Institute of Fungal Resources of Guizhou University (GZUIFR). The fungal strains were isolated on potato dextrose agar (PDA) medium; one strain was isolated from part of the body and the second strain was isolated from the synnemata.

### Strain culture and identification

The isolated strains were inoculated on PDA at 25 °C for 14 d under 12-h light/12-h dark conditions. The fresh hyphae were observed with an optical microscope (OM, BK5000, OPTEC, USA) following pretreatment with lactophenol cotton blue solution or normal saline.

### DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted using a previously described method (Chiriví-Salomón et al. 2015, Zou et al. 2016). The primers used for PCR amplification of the ITS region, *SSU*, *LSU*, *TEF*, *RPB1* and *RPB2* are listed in Table 1. The PCR reaction conditions employed for each genetic region followed those used in the references listed in Table 1.

To conduct phylogenetic analysis of the sequences obtained, sequences for selected taxa based on recent phylogenetic studies of *Lecanicillium* (Chen et al. 2017, Huang et al. 2018) and Cordycipitaceae (Sung et al. 2007, Kepler et al. 2017, Mongkolsamrit et al. 2018) were downloaded from the National Center for Biotechnology Infor-

**Table 1.** Primer information and provenance in this study.

| Gene | Primer | Provenance |
|---|---|---|
| ITS | F: 5'-TCCGTAGGTGAACCTGCGG-3' | White et al. 1990 |
| | R: 5'-TCCTCCGCTTATTGATATGC-3' | |
| *SSU* | F: GTAGTCATATGCTTGTCTC | White et al. 1990 |
| | R: CTTCCGTCAATTCCTTTAAG | |
| *LSU* | F: GTTTCCGTAGGTGAACCTGC | Curran et al. 1994 |
| | R: ATATGCTTAAGTTCAGCGGGT | |
| *TEF* | F: 5'-GCCCCCGGCCATCGTGACTTCAT-3' | van den Brink et al. 2011 |
| | R: 5'-ATGACACCGACAGCGACGGTCTG-3' | |
| *RPB1* | F: 5'-CCWGGYTTYATCAAGAARGT-3' | Castlebury et al. 2004 |
| | R: 5'-CAYCCWGGYTTYATCAAGAA-3' | |
| *RPB2* | F: 5'-GACGACCGTG ATCACTTTGG-3' | van den Brink et al. 2011 |
| | R: 5'-CCCATGGCCTGTTTGCCCAT-3' | |

mation GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). A total of 79 accessions of Cordycipitaceae were selected for this study. The sequences used in the study are listed in Table 2.

## Sequence alignment and phylogenetic analyses

The DNA sequences used in this study were edited using the LASERGENE software (version 6.0; DNASTAR, Madison, WI, USA). Multiple sequence alignments for *TEF*, *RPB1* and *RPB2* were performed in MAFFT (Katoh and Standley 2013) with the default settings. Multiple sequence alignments for ITS, *LSU* and *SSU* were conducted using MUSCLE algorithm (Edgar 2004) from MEGA 6 (Tamura et al. 2013). The sequences were edited manually. A multiple alignment of the combined partial ITS+*SSU*+*LSU*+*TEF*+*RPB1*+*RPB2* sequences were assembled with MEGA 6 (Tamura et al. 2013) and SEQUENCEMATRIX 1.7.8 (Vaidya et al. 2011). The command 'hompart' in PAUP* 4.0b10 was used for assessment of concordance amongst the genes and the ITS region (Swofford 2001). Bayesian inference (BI) was performed using MRBAYES 3.2 (Ronquist et al. 2012) and maximum likelihood (ML) analysis was performed using RAxML (Alexandros 2014) to analyse the combined data which were divided into twelve separate partitions (Kepler et al. 2017; Mongkolsamrit et al. 2018). Two maximum likelihood (ML) analysis and Bayesian inference (BI) analysis were performed. The first analysis was performed as reported by Huang et al. (2018), using the *Simplicillium lanosoniveum* as the outgroup. The second analysis was performed with *Akanthomyces*, *Samsoniella*, *Blackwellomyces*, *Hevansia*, *Simplicillium*, all the *lecanicillium* and use of *Beauveria* as outgroup (Mongkolsamrit et al. 2018). Nucleotide substitution models were determined by MrModeltest 2.3 (Nylander 2004). For BI, 10 000 000 generations were performed with one tree selected every 500th generation and the GTR+I+G evolutionary model was used. For ML, the model GTRGAMMA was used and a bootstrap analysis with 500 replicates was performed to assess statistical support for the tree topology. Phylogenetic trees were viewed with TREEGRAPH.

**Table 2.** Specimen information and GenBank accession numbers used in this study.

| Species | Voucher Information | ITS | *SSU* | *LSU* | *TEF* | *RPB1* | *RPB2* |
|---|---|---|---|---|---|---|---|
| *Akanthomyces waltergamsii* | TBRC 7250 | MF140749 | | MF140715 | MF140835 | | |
| *A. waltergamsii* | TBRC 7251 | MF140747 | | MF140713 | MF140833 | MF140781 | MF140805 |
| *A. sulphureus* | TBRC 7248 | MF140758 | | MF140722 | MF140843 | MF140787 | MF140812 |
| | TBRC 7249 | MF140757 | | MF140721 | MF140842 | MF140786 | MF140734 |
| *A. thailandicus* | TBRC 7246 | MF140755 | | MF140719 | MF140840 | | MF140810 |
| | TBRC 7245 | MF140754 | | | MF140839 | | MF140809 |
| *A. kanyawimiae* | TBRC 7242 | MF140751 | | MF140718 | MF140838 | MF140784 | MF140808 |
| | TBRC 7244 | MF140752 | | MF140716 | MF140836 | | |
| *A. aculeatus* | HUA 186145 | | MF416572 | MF416520 | MF416465 | | |
| *A. pistillariaeformis* | HUA 186131 | | MF416573 | MF416521 | MF416466 | | |
| *A. coccidioperitheciatus* | NHJ 6709 | JN049865 | EU369110 | EU369042 | EU369025 | EU369067 | EU369086 |
| *A. aculeatus* | TS 772 | KC519371 | KC519368 | KC519370 | KC519366 | | |
| *A. tuberculatus* | BCC16819 | | MF416600 | MF416546 | MF416490 | MF416647 | MF416490 |
| *Ascopolyporus villosus* | ARSEF 6355 | | | AY886544 | DQ118750 | DQ127241 | |
| *Asc. polychrous* | P.C. 546 | | | DQ118737 | DQ118745 | DQ127236 | |
| *Beauveria bassiana* | ARSEF 1564 | HQ880761 | | | HQ880974 | HQ880833 | HQ880905 |
| *Bea. brongniartii* | BCC 16585 | JN049867 | JF415951 | JF415967 | JF416009 | JN049885 | JF415991 |
| *Blackwellomyces cardinalis* | OSC 93610 | JN049843 | AY184974 | AY184963 | EF469059 | EF469088 | EF469106 |
| *Bla. cardinalis* | OSC 93609 | | AY184973 | AY184962 | DQ522325 | DQ522370 | DQ522422 |
| *Bla. pseudomilitaris* | NBRC 101409 | JN943305 | JN941748 | JN941393 | | JN992482 | |
| | NBRC 101410 | JN943307 | JN941747 | JN941394 | | JN992481 | |
| *Gibellula longispora* | NHJ 12014 | | EU369098 | | EU369017 | EU369055 | EU369075 |
| *Gibellula* sp. | NHJ 7859 | | EU369107 | | | EU369064 | EU369085 |
| | NHJ 10788 | | EU369101 | EU369036 | EU369019 | EU369058 | EU369078 |
| | NHJ 5401 | | EU369102 | | | EU369059 | EU369079 |
| *G. ratticaudata* | ARSEF 1915 | JN049837 | DQ522562 | DQ518777 | DQ522360 | DQ522408 | DQ522467 |
| *Hevansia nelumboides* | BCC 41864 | JN201871 | JN201863 | JN201873 | JN201867 | | |
| *Hev. novoguineensis* | NHJ 11923 | | EU369095 | EU369032 | EU369013 | EU369052 | EU369072 |
| *Hev. arachnophila* | NHJ 10469 | | EU369090 | EU369031 | EU369008 | EU369047 | |
| *Hev. cinerea* | NHJ 3510 | | EU369091 | | EU369009 | EU369048 | EU369070 |
| *Lecanicillium acerosum* | CBS418.81 | EF641893 | KM283762 | KM283786 | KM283810 | KM283832 | KM283852 |
| *L. antillanum* | CBS350.85 | AJ292392 | AF339585 | AF339536 | DQ522350 | DQ522396 | DQ522450 |
| *L. aphanocladii* | CBS797.84 | | KM283763 | KM283787 | KM283811 | KM283833 | KM283853 |
| *L. aranearum* | CBS726.73a | AJ292464 | AF339586 | AF339537 | EF468781 | EF468887 | EF468934 |
| *L. araneicola* | BTCC-F35 | AB378506 | | | | | |
| *L. araneogenum* | GZU1031Lea | | KX845705 | KX845703 | KX845697 | KX845699 | KX845701 |
| *L. attenuatum* | CBS402.78 | AJ292434 | AF339614 | AF339565 | EF468782 | EF468888 | EF468935 |
| | KACC42493 | | KM283756 | KM283780 | KM283804 | KM283826 | KM283846 |
| ***L. cauligalbarum*** | **GZUIFRZHJ01** | **MH730663** | **MH730665** | **MH730667** | **MH801920** | **MH801922** | **MH801924** |
| | **GZUIFRZHJ02** | **MH730664** | **MH730666** | **MH730668** | **MH801921** | **MH801923** | **MH801925** |
| *L. dimorphum* | CBS345.37 | | KM283764 | KM283788 | KM283812 | KM283834 | KM283854 |
| *L. flavidum* | CBS300.70D | EF641877 | KM283765 | KM283789 | KM283813 | | KM283855 |
| *L. fungicola var. aleophilum* | CBS357.80 | NR_111064 | KM283767 | KM283791 | KM283815 | KM283835 | KM283856 |
| *L. fungicola var. fungicola* | CBS992.69 | NR_119653 | KM283768 | KM283792 | KM283816 | | KM283857 |
| *L. fusisporum* | CBS164.70 | AJ292428 | KM283769 | KM283793 | KM283817 | KM283836 | KM283858 |
| *L. kalimantanense* | BTCC-F23 | AB360356 | | | | | |
| *L. lecanii* | CBS101247 | JN049836 | KM283770 | KM283794 | DQ522359 | KM283837 | KM283859 |
| | CBS102067 | | KM283771 | KM283795 | KM283818 | KM283838 | KM283860 |
| *L. longisporum* | CBS102072 | | KM283772 | KM283796 | KM283819 | KM283839 | KM283861 |
| | CBS126.27 | | KM283773 | KM283797 | KM283820 | KM283840 | KM283862 |
| *L. muscarium* | CBS143.62 | | KM283774 | KM283798 | KM283821 | KM283841 | KM283863 |

| Species | Voucher Information | ITS | *SSU* | *LSU* | *TEF* | *RPB1* | *RPB2* |
|---|---|---|---|---|---|---|---|
| *L. nodulosum* | IMI 338014R | EF513012 | EF513075 | | | | |
| *L. pissodis* | CBS118231 | | KM283775 | KM283799 | KM283822 | KM283842 | KM283864 |
| *L. primulinum* | JCM 18525 | AB712266 | | AB712263 | | | |
| | JCM 18526 | AB712267 | | AB712264 | | | |
| *L. psalliotae* | CBS532.81 | JN049846 | AF339609 | AF339560 | EF469067 | EF469096 | EF469112 |
| | CBS101270 | | EF469128 | EF469081 | EF469066 | EF469095 | EF469113 |
| | CBS363.86 | | AF339608 | AF339559 | EF468784 | EF468890 | |
| *L. restrictum* | CCF5252 | LT548279 | | | LT626943 | | |
| *L. sabanense* | JCHA5 | KC633232 | KC633251 | KC875225 | KC633266 | | KC633249 |
| *L. saksenae* | IMI 179841 | AJ292432 | | | | | |
| *L. subprimulinum* | HKAS99548 | MG585314 | MG585316 | MG585315 | MG585317 | | |
| | HKAS99549 | MG585318 | MG585320 | MG585319 | MG585321 | | |
| *L. testudineum* | UBOCC-A112180 | LT992874 | | | LT992868 | | |
| | UBOCC-A116026 | LT992871 | | | LT992867 | | |
| *L. tenuipes* | CBS309.85 | JN036556 | KM283778 | KM283802 | DQ522341 | KM283844 | KM283866 |
| *L. uredinophilum* | KACC44082 | | KM283758 | KM283782 | KM283806 | KM283828 | KM283848 |
| | KACC47756 | | KM283759 | KM283783 | KM283807 | KM283829 | KM283849 |
| *L. wallacei* | CBS101237 | EF641891 | AY184978 | AY184967 | EF469073 | EF469102 | EF469119 |
| *Samsoniella inthanonensis* | TBRC 7915 | MF140761 | | MF140725 | MF140849 | MF140790 | MF140815 |
| *Sam. inthanonensis* | TBRC 7916 | MF140760 | | MF140724 | MF140848 | MF140789 | MF140814 |
| *Sam. aurantia* | TBRC 7271 | MF140764 | | MF140728 | MF140846 | MF140791 | MF140818 |
| | TBRC 7272 | MF140763 | | MF140727 | MF140845 | | MF140817 |
| *Sam. alboaurantium* | CBS 240.32 | AY624178 | JF415958 | JF415979 | JF416019 | JN049895 | JF415999 |
| | CBS 262.58 | MH857775 | | MH869308 | JQ425685 | MF416654 | MF416448 |
| *Simplicillium lamellicola* | CBS 116.25 | AJ292393 | AF339601 | AF339552 | DQ522356 | DQ522404 | DQ522462 |
| *Sim. lanosoniveum* | CBS 704.86 | AJ292396 | AF339602 | AF339553 | DQ522358 | DQ522406 | DQ522464 |
| | CBS 101267 | AJ292395 | AF339603 | AF339554 | DQ522357 | DQ522405 | DQ522463 |
| *Sim. obclavatum* | CBS 311.74 | | AF339567 | AF339517 | EF468798 | | |

## Results

### Sequencing and phylogenetic analysis

The first sequence dataset consisted of 3793 bases, including inserted gaps (ITS: 506 bp; *SSU*: 579 bp; *LSU*: 490 bp; *TEF*: 772 bp; *RPB1*: 561 bp; *RPB2*: 885 bp). The second sequence dataset consisted of 2944 bases, including inserted gaps (ITS: 526 bp; *SSU*: 456 bp; *LSU*: 409 bp; *TEF*: 386 bp; *RPB1*: 500 bp; *RPB2*: 667 bp). No significant differences in topology were observed between the BI and ML phylogenies. The first tree formed with almost all the *Lecanicillium* species (only *Lecanicillium evansii* could not be found in the NCBI) and one *Simplicillium* species (*Simplicillium lanosoniveum*). The phylogeny was resolved into 4 clades obviously. *Lecanicillium cauligalbarum* formed an independent branch in a polytomy together with a clade containing *L. flavidum* and *L. fungicola* and a major clade consisting of 27 accessions. The *L. cauligalbarum* lineage received maximum statistical support (BI posterior probabilities 1, ML boostrap 100%), which still remains unnamed (Figure 1). In the second tree, the four *Lecanicillium* clades were also be supported. *Lecanicillium cauligalbarum* formed an independent branch in a polytomy together with a clade containing *Blackwellomyces cardinalis* and *Blackwellomyces pseudomilitaris* (BI posterior probabilities 1, ML boostrap 85%) (Figure 2).

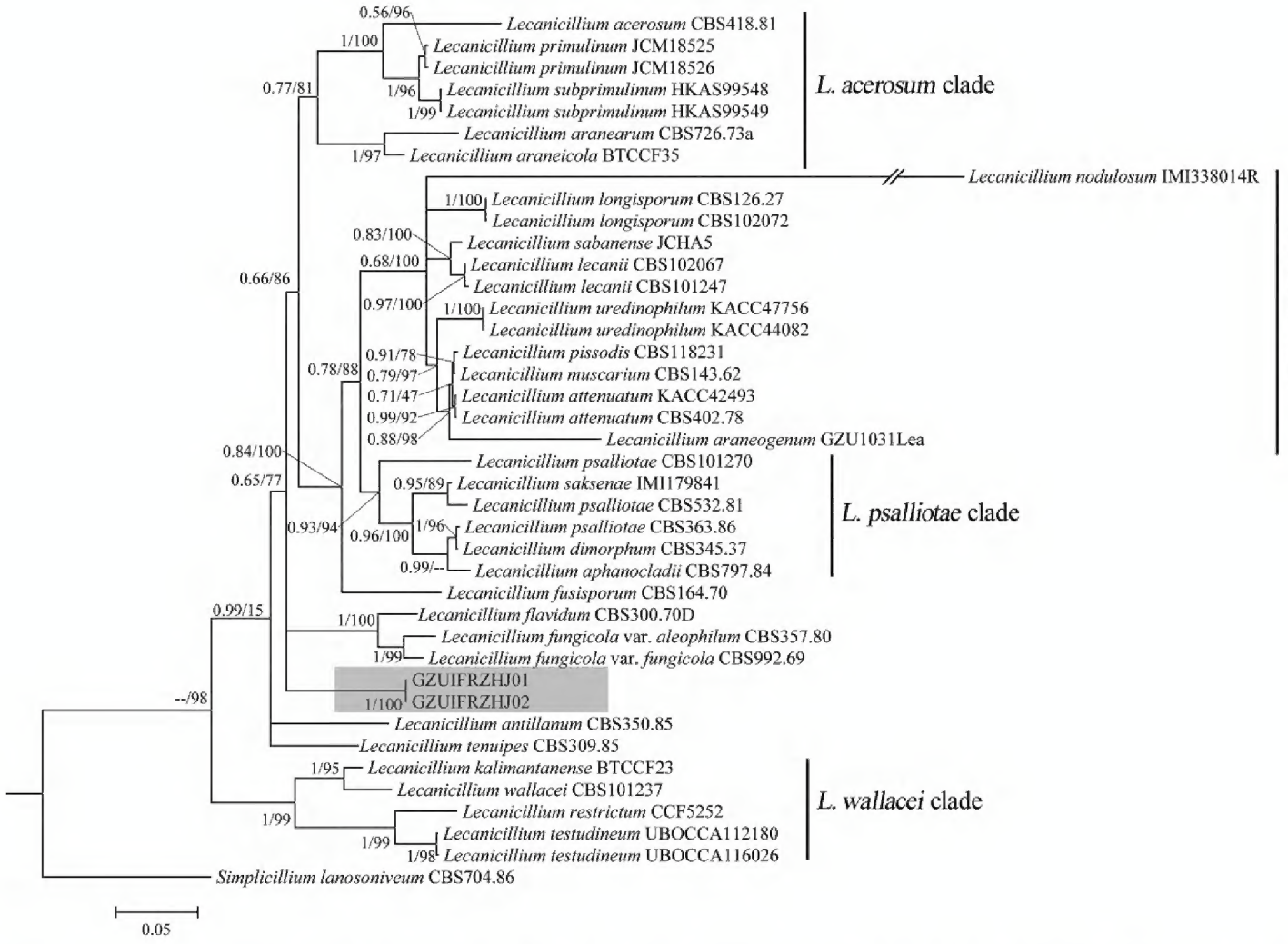


**Figure 1.** Phylogenetic analysis of the isolated strains GZUIFRZHJ01 and GZUIFRZHJ02 and related species derived from a combined dataset of partial ITS+*SSU*+*LSU*+*TEF*+*RPB1*+*RPB2* sequences. Statistical support values (≥ 0.5/50%) are shown at the nodes for BI posterior probabilities/ML boostrap support.

## Taxonomy

***Lecanicillium cauligalbarum* X. Zou, J.R. Zhi & Y.M. Zhou, sp. nov.**
MycoBank: MB827984
Figure 3

**Diagnosis.** Characterised by phialides gradually tapering towards the apex, solitary or 2–3 whorls, 9–14.4 × 1.4–1.8 μm. Conidia cylindric, aseptate, 3.6–6.3 × 0.9–1.8 μm.

**Type.** CHINA, Guizhou Province, Sandu county (107°53', 107°58'E; 24°54', 25°59'N, approximately 560–1365 m above sea level), September 2015, Yeming Zhou & Xiao Zou. Sequences from isolated strains (GZUIFRZHJ01 and GZUIFRZHJ02) have been deposited in GenBank (accession numbers to be provided).

**Description.** Colony on PDA 15 mm in diameter after 7 days, 33 mm in diameter after 14 days at 25 °C, colony circular, white, cottony, umbonate, with radiating surface texture from above, with clear radial crack and primrose-yellow from reverse. Mycelium 0.9–1.8 μm wide, hyaline, smooth, septated, branched. Conidiophores usually arising from aerial hyphae, sporulate abundant. Phialides gradually tapering towards the apex, solitary or 2–3 whorls, 9–14.4 × 1.4–1.8 μm. Conidia cylindric, aseptate, 3.6–6.3 × 0.9–1.8 μm. In culture, both phialides and conidia are of similar general shape and size to those found on the host stemborer.

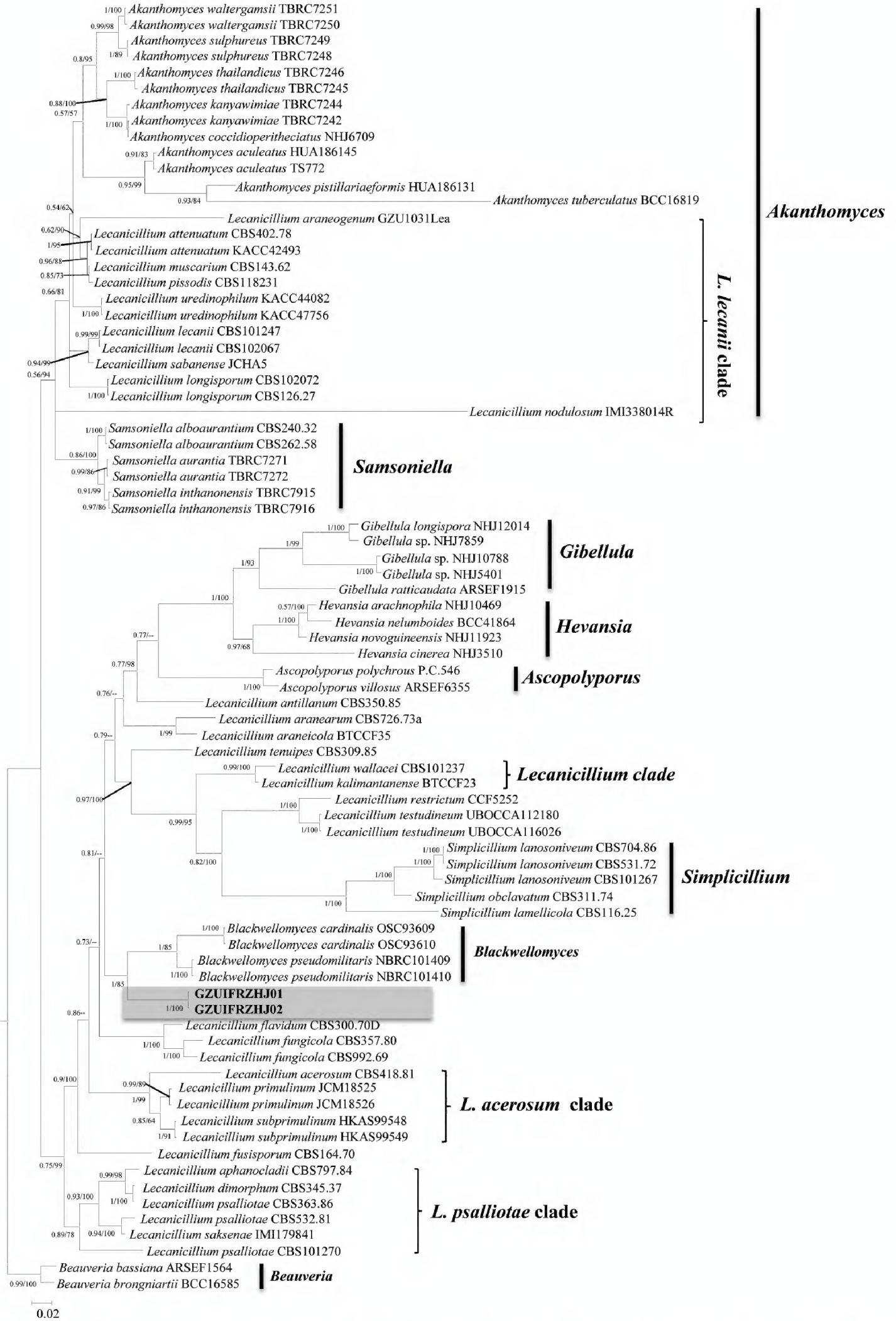


**Figure 2.** Phylogenetic relationships of the form genus *Lecanicillium*, *Akanthomyces*, *Samsoniella*, *Blackwellomyces*, *Hevansia* and related genera in the Cordycipitaceae. Statistical support values (≥ 0.5/50%) are shown at the nodes for BI posterior probabilities/ML boostrap support.

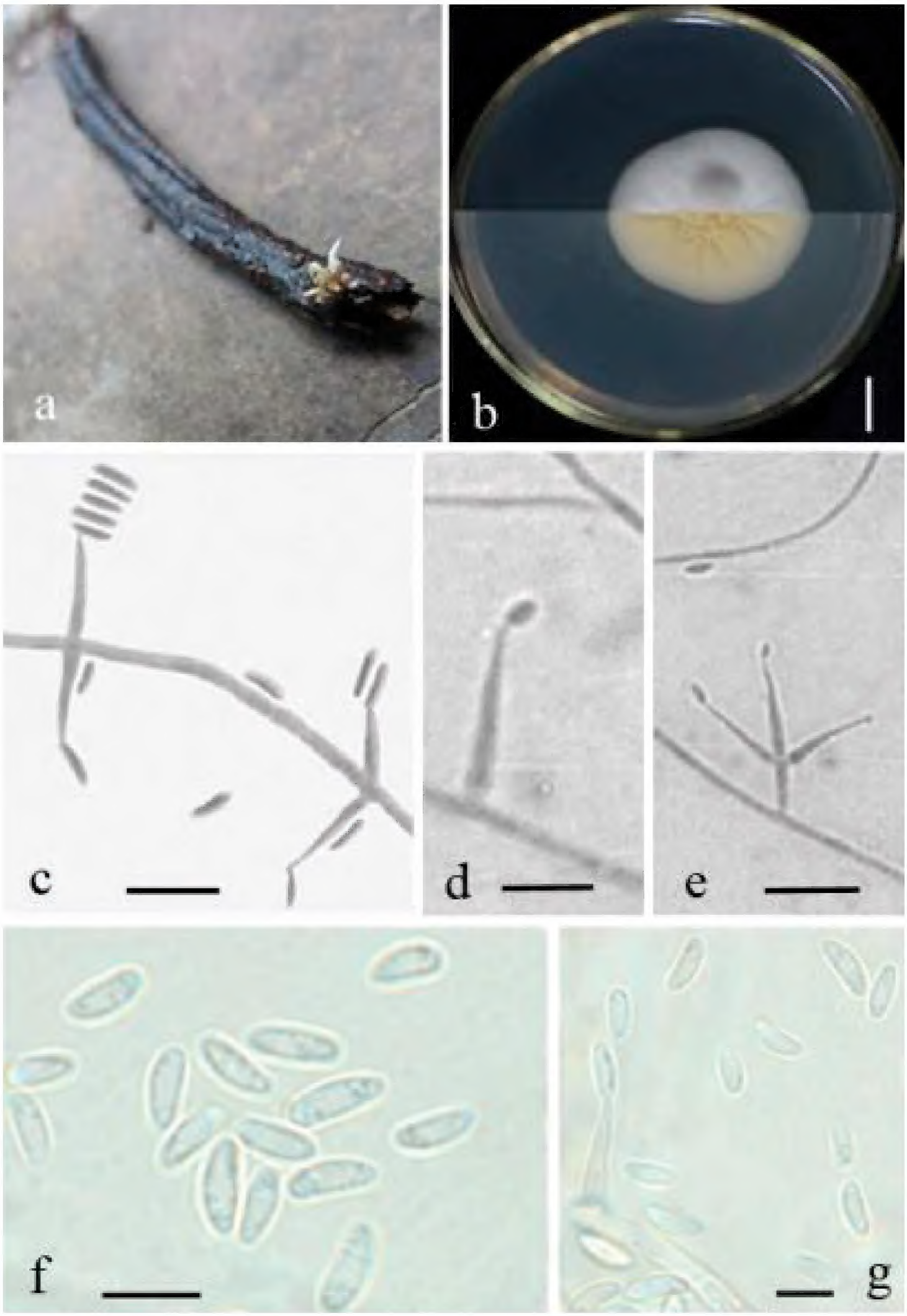


**Figure 3.** *Lecanicillium cauligalbarum*. **a** Synnemata emerged from the corpse of a stemborer (Lepidoptera) **b** Culture plate, showing the front (upper) and the back (lower) of the colony, cultured on PDA medium **c–e** Phialides solitary or in 2–3 whorls **f–g** Conidia. Scale bars: 10 mm (**b, c, e**), 5 μm (**d, f, g**).

**Host.** Stemborer (Lepidoptera) hidden amongst wooden sticks.

**Habitat and distribution.** Hidden amongst pieces of wood in humid forests of southwest China.

**Etymology.** The epithet '*cauligalbarum*' refers to the host (stemborer).

**Teleomorph.** Not known.

**Remarks.** With regard to phylogenetic relationships, *L. cauligalbarum* is closely related to the *L. fungicola* clade and *L. fusisporum*. The two strains (GZUIFRZHJ01 and GZUIFRZHJ02) formed a distinct lineage. All *Lecanicillium* species were included in the phylogenetic analysis except for *L. evansii* for which sequence data could not be located in public databases, although Zare and Gams (2001) published ITS sequences. The morphological features of *L. evansii* include brownish-cream to brown reverse, phialides solitary or up to 3–4 per node and two types of the conidia, slightly falcate with a pointed end macroconidia 4.5–7.5 × 0.8–1.2 μm and slightly curved microconidia 2.0–3.0 × 0.8–1.2 μm (Zare and Gams 2001). *L. evansii* is distinct from *L. cauligalbarum*, which has conidia of 3.6–6.3 × 0.9–1.8 μm and 9–14.4 × 1.4–1.8 μm phialides.

In morphology *L. cauligalbarum*is is similar to *L. aphanocladii*, *L. attenuatum* and *L. nodulosum* with regard to the short conidiogenous cell (Table 3). However, *L. cauligalbarum* is distinguished by the pattern of spore production and the frequency of the wheel structure.

**Table 3.** Morphological comparison among *Lecanicillium cauligalbarum* and the other related species.

| Species | Colonies | Conidiogenous cell | Conidia | Refrence |
|---|---|---|---|---|
| *Lecanicillium acerosum* | White, yellow reverse | Solitary or up to 4–5, 30–32×1.8–2.2 μm | Macroconidia fusiform, straight to slightly falcate, 15–20×1.6–2.2 μm, microconidia fusiform, 4.5–7.5×1.0–1.5 μm | Zare and Gams 2001 |
| *L. antillanum* | White, cream-coloured reverse | Solitary or up to 6, subulate, 18–31×1 μm (at the top) | Macroconidia fusiform, 11–18×0.8–1.5 μm, microconidia ellipsoidal, 3–4×0.8–1.2 μm | Zare and Gams 2001 |
| *L. aphanocladii* | White, red, reddish-white to cream-coloured reverse | Solitary, in pairs, verticillate, flask-shaped in the beginning, tapering into a thread-like neck, 4.5–11×1.0–1.8 μm | Solitary, oval to sub-globose, 2.7–4×1.5–2.2 μm | Zare and Gams 2001 |
| *L. aranearum* | White, yellowish-cream reverse | Tapering towards the apex, 20–30×1.2–1.5 μm | Straight or curved, usually asymmetrically narrowed or subacute at the ends, 5–8×0.7–1.5 μm | Zare and Gams 2001 |
| *L. araneicola* | White, creamy-white reverse | Solitary or in whorls of 2–4, slender, tapering toward the tip, (14-)19–31.5×1–2 μm | Macroconidia slightly curved to nearly straight, (7.5-)8.5–12(-14)×1.5–2 μm, microconidia allantoid to ellipsoidal with round ends, 3–5×1–2 μm | Sukarno et al. 2009 |
| *L. araneogenum* | White to light grey, light yellow reverse | Produced in whorls of (1-)2–6(-8), 30–64×1.1–3.2 μm | Forming mostly globose heads, cylindric, 3.2–8.6×1.3–1.6 μm | Chen et al. 2017 |
| *L. attenuatum* | White, yellowish-white reverse | Up to 3–5 per node, 9–15.5×1–2 μm | Cylindrical with attenuate base, 4.5–6.5×1.5–2.0 μm | Zare and Gams 2001 |
| ***L. cauligalbarum*** | White, primrose-yellow reverse | Solitary or 2–3 whorls, 9–14.4×1.4–1.8 μm | Cylindrical, 3.6–6.3×0.9–1.8 μm | **This work** |
| *L. dimorphum* | White, cream to brownish-cream, red reverse | Two kinds: solitary or 4–5 whorls, 14–30×1.0–1.5μm; short, 5–12×0.7–1.5 μm | Macroconidia falcate with sharply pointed ends, usually evenly curved, 6–11×1.5–2.5μm, microconidia oval to ellipsoidal, 2.5–4.5×1.0–1.5 μm | Zare and Gams 2001 |
| *L. evansii* | White, creamy, brownish-cream to brown reverse, | Solitary or up to 3–4 per node, 20–45×1–1.2 μm | Macroconidia slightly falcate, 4.5–7.5×0.8–1.2μm, microconidia ellipsoidal or curved, 2.0–3.0×0.8–1.2 μm | Zare and Gams 2001 |

| Species | Colonies | Conidiogenous cell | Conidia | Refrence |
|---|---|---|---|---|
| *L. flavidum* | Greyish-white to citron-yellow, citron-yellow reverse | In whorls, 12–35×1.5–2.5 µm, 0.5–1 µm at the tips | Mostly fusiform, long-ellipsoidal to almost cylindrical, slightly sickle-shaped, 4–8×1.5–2 µm | Zare and Gams 2008 |
| *L. fungicola* var. *aleophilum* | White, reverse uncoloured | Whorls of 3–10, 15–30×1.5–2.5 µm, 0.5–1.5 µm at the tips | Oblong, fusiform, long ellipsoidal to almost cylindrical, irregular size, 4.5–8×1–2.5 µm | Zare and Gams 2008 |
| *L. fungicola* var. *fungicola* | Dirty white, reverse uncoloured | Whorls of 3–7, 14–20(-45)×1.5–3µm, 0.5–1 µm at the tip | Fusiform, long-ellipsoidal to almost cylindrical, sickle-shaped, very unequal size, 4–9(-12)×1.5–2.5(-3.5) µm | Zare and Gams 2008 |
| *L. fusisporum* | White, with red reverse and pigment diffusing | Solitary or up to 5, 16–26×1.0–1.5 µm | Fusiform, straight and rather broad, 3–5×1.5–2.0 µm | Zare and Gams 2001 |
| *L. kalimantanense* | White, creamy-white reverse | Solitary or more often in whorls of 2–5, slender, tapering toward the apex, 12.5–36×1–2 µm | Acerose to fusoid with pointed ends, slightly curved, of varying size, (3.5-)4.5–12×1–2 µm | Sukarno et al. 2009 |
| *L. lecanii* | Yellowish-white, deep yellow reverse | Aculeate and strongly tapering, singly or up to 6, 11–20(-30)×1.3–1.8 µm | Typically short-ellipsoidal, 2.5–3.5(-4.2)×1–1.5 µm, homogeneous in size and shape | Zare and Gams 2001 |
| *L. longisporum* | White to sulphur-yellow, cream-coloured to pale yellow reverse | Tapering towards the apex(sub-aculeate), singly or up to 5–6 or on secondary phialides, 20–40×1.2–2.7 µm | Produced in globose heads, ellipsoidal to oblong-oval, 5.0–10.5×1.5–2.5 µm | Zare and Gams 2001 |
| *L. muscarium* | White, cream-coloured or uncoloured reverse | Solitary or up to 6 (less frequent than in *L. lecanii*), (15-)20–35×1–1.5 µm | Produced in globose heads, ellipsoidal to subcylindrical, more irregular in size and shape, (2-)2.5–5.5(-6)×1–1.5(-1.8) µm | Zare and Gams 2001 |
| *L. nodulosum* | White, cream-coloured reverse | Subulate, up to 6, 10–20×1.5 µm | Produced in heads of about 10µm diam., oval, 2.5–4.5×1.2–1.5 µm | Zare and Gams 2001 |
| *L. pissodis* | White, ceram to yellow reverse | Solitary, up to 3, 16-(18–28)-38×1–2 µm | Up to more than 50 formed in globose droplets, cylindrical to oval, very variable in size and shape, 4–9.2×1.6–2.4 µm | Kope and Leal 2006 |
| *L. primulinum* | Pale yellow, yellowish-brown reverse, brownish-yellow pigment | Solitary or in whorls of 2–5, tapering toward the tip, 20–50(-85)×0.8–1.8 µm | Macroconidia ellipsoidal to cylindrical, 5.0–9.5×1.2–2.5 µm, microconidia oval to ellipsoidal, 3.0–4.8×1.0–2.5 µm | Kaifuchi et al. 2013 |
| *L. psalliotae* | White and red, reddish-cream to cream-coloured reverse, red to purple pigment | Aculeate, solitary or more often 3–4(-6) in whorls on each node, 25–35×1.0–1.5 µm | Macroconidia curved, falcated, 5–10×1.2–1.7 µm, microconidia oval or ellipsoidal, 2.7–3.7×1–1.5 µm | Zare and Gams 2001 |
| *L. restrictum* | Yellowish-white, reverse yellowish-white to pale yellow | Solitary or in whorls of 2–5, tapering toward the tip, (12-)17–30(-36)×0.5–1.5 µm, 0.3–0.5 µm wide on the tip | Macroconidia fusiform or slightly falcate, (5-)6–10(-12)×1–1.5 µm, microconidia ovate, ellipsoidal, obovate or fusoid, frequently slightly curved, 2.5–3×1–1.5 µm | Crous et al. 2018 |
| *L. sabanense* | Pale yellow to duller yellow, orange reverse | Solitary or in whorls of 2–4, 13–19×1.0–2.0 µm, gradually tapering to 0.5–1.0 µm | Forming mostly globose heads, 9–20 µm diam, ellipsoidal to ovoid, 3.5–4.5×1.5–2.0 µm | Chiriví-Salomón et al. 2015 |
| *L. saksenae* | White, creamy white reverse | Solitary or often in whorls of 2–4, slender, tapering towards the apex, 14.5–36×1.0–2.0 µm | Macroconidia slightly curved, 6–13×1.5–2 µm, microconidia ellipsoidal to fusoid with round ends, nearly straight to slightly curved, 2.5–5×1.5–2 µm | Sukarno et al. 2009 |
| *L. subprimulinum* | Creamy, primrose-yellow reverse | Tapering towards apex, discrete, solitary or up to 2–3 per node, 19–32×1.5–3.5 µm | Ovoid to ellipsoidal, elongated, straight or slightly curved, 4–15×2–6 µm | Huang et al. 2018 |
| *L. testudineum* | White, centrally raised, wrinkled, reverse pale yellow to greyish-yellow | Solitary or in whorls of 2–4, tapering toward the tip, (13-)16–45(-53)×0.5–1 µm (exceptionally 80 µm long), 0.5–1 µm wide on the tip | Macroconidia fusiform or slightly falcate, 3.5–6(-6.5)×1–1.5 µm, microconidia ovate, ellipsoidal or fusoid, curved to reniform, 2–3.5×1–1.5 µm | Crous et al. 2018 |
| *L. tenuipes* | White, reverse uncoloured | Arising singly or in scanty whorls, 20–35(-40)×1.2–1.5 µm | Microconidia ellipsoidal, straight, 3.0–5.5(-6.5)×1.0–1.5 µm, microconidia fusiform to falcate, 8–17×1.5–1.8 µm | Gams et al. 1984; Zare and Gams 2001 |
| *L. uredinophilum* | White to cream coloured, reverse cream coloured | Produced singly or in whorls of up to 3–5, 20–60×1–2.5(-3) µm | Cylindric, oblong or ellipsoid, 3–9×1.8–3 µm | Park et al. 2016 |
| *L. wallacei* | White, cream-coloured to creamish-brown reverse | Sollitary or up to 3–4, aculeate, (14-)17–25(29)×0.7–1.2 µm | Macroconidia, fusiform to falcate, (7.0-)8.5–10.5(-12.5)×1.0–1.5 µm, microconidia ellipsoidal to slightly falcate, (3.0-)4.0–5.5(-6.5)×0.7–1.2 µm | Zare and Gams 2001, 2008 |

## Discussion

The genera *Lecanicillium* and *Simplicillium* belong to the Cordycipitaceae (Sung et al. 2007). The two genera are indistinguishable in morphological traits (Sung et al. 2001; Zare and Gams 2001). However, *Lecanicillium* and *Simplicillium* are clearly separated in molecular phylogenetic analyses (Kouvelis et al. 2008; Maharachchikumbura et al. 2015; Nonaka et al. 2013). As an insect pathogen, *Lecanicillium* spp. has potential for development as effective biological control agents against a number of plant diseases, insect pests and plant-parasitic nematodes (Goettel et al. 2008). Fifteen commercial preparations based on *Lecanicillium* spp. have been developed or are in the process of being developed (Faria and Wraight 2007). Kepler et al. (2017) concluded that *Lecanicillium* should be incorporated into *Akanthomyces* and formally transferred a number of *Lecanicillium* species. However, the compatibility of *Lecanicillium* was not so good in this study. Species that have been transferred to *Akanthomyces* were all assembled in the *L. lecanii* clade in the present study. The remaining species included in the present analyses were divided into multiple clades similar to those retrieved by Kepler et al. (2017). Relationships amongst *Lecanicillium* species thus appear to be more complicated than expected. Thus, we also prefer to describe the new taxon as a *Lecanicillium* species, consistent with Huang et al. (2018), owing to the uncertainty in generic boundaries.

In a comparison of all *Lecanicillium* species included in the present study, we were unable to identify morphological synapomorphies that characterise the phylogenetic groups. However, the species that show a close phylogenetic relationship are more similar in morphology than those that are phylogenetically distant. For example, the *L. lecanii* clade, which has globose heads with a higher number of conidia, are distinguishable from those clades that usually have one conidium visible at the top of the phialide in the phylogenetic tree presented here. In our phylogeny study, the node connecting *L. antillanum* and *L. tenuipes* is the basal node for the major clade. So the relationships of all of the lineages involved may change with more data or a different dataset. Therefore, more species are needed to enrich the phylogenetic study of *Lecanicillium* spp.

We know that *Lecanicillium* has a different origin into the Cordycipitaceae. We consider that the ones '*L. lecanii* clade' in pig.1 form a strong clade inside of *Akanthomyces*. Maybe all these should be moved to the *Akanthomyces* including *Lecanicillium longisporum*. In addition, the elimination of the genus may create more chaos considering the unsolved other clades.

*Blackwellomyces* Spatafora & Luangsa-ard is diagnosed by the unique characters of the ascospore, which have irregularly spaced septa and do not disarticulate into partspores at maturity as advised by Kepler et al. (2017). It includes *Blackwellomyces cardinalis* and *Blackwellomyces pseudomilitaris*. Asexual morphs have been described as similar to species in *Clonostachys*, *Hirsutella*, *Isaria* and *Mariannaea* (Hywel-Jones 1994; Sung and Spatafora 2004). Although the new species are close to the *Blackwellomyces* in the phylogenetic tree, we think they are clearly distinguished from *Blackwellomyces* by

the morphology. We also treat the new species as *Lecanicillium* considering the small sample and the unknown teleomorph.Thus, based on the present molecular phylogeny, derived from nuclear and ribosomal DNA sequence data, together with morphological evidence, a distinct new *Lecanicillium* species, *L. cauligalbarum*, is proposed.

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